CHEMICAL
RESEARCH,
DEVELOPMENT &
ENGINEERING
CENTER

CRDEC-TR-139

CHEMICAL INHIBITION OF RABBIT SPERM CELL MOTILITY IN TOXICOLOGICAL TESTING

Ronald J. Young, Ph.D.
David Burnett

RESEARCH DIRECTORATE

January 1990

U.S. ARMY
ARMAMENT
MUNITIONS
CHEMICAL COMMAND

Aberdeen Proving Ground, Maryland 21010-5423

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

Distribution Statement

Approved for public release; distribution is unlimited.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) CRDEC-TR-139	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION CRDEC 6b. OFFICE SYMBOL (if applicable) SMCCR-RST-C	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) Aberdeen Proving Ground, MD 21010-5423	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION CRDEC 8b. OFFICE SYMBOL (if applicable) SMCCR-RST-C	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Aberdeen Proving Ground, MD 21010-5423	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. / PROJECT NO. 1L161101 / TASK NO. A91A / WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)

Chemical Inhibition of Rabbit Sperm Cell Motility in Toxicological Testing

12. PERSONAL AUTHORS (S)

Young, Ronald J., Ph.D., and Burnett, David

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Technical	FROM 87 Apr TO 87 Sep	1990 January	13

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Rabbit, Sperm, Cell, Cytotoxic, Cell Motility
06	03, 11		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The use of motile rabbit spermatozoa to assess the potential cytotoxic effect of chemicals was investigated. A suspension of motile sperm cells in a defined medium was incubated at 37°C under an atmosphere of 5% CO_2, 8% O_2, and 87% N_2 with three different concentrations of the test chemicals. At specified time intervals, the motion characteristics of the cells were observed by phase microscopy (100x). The test chemicals were ranked in order of their ability to inhibit rabbit cell motility. There was a good correlation between this ranking, the performance of the chemicals in the Draize test, and the inhibition by the chemicals of the in vitro incorporation of amino acids and uridine by mouse 3T3 cells. The inhibition of rabbit sperm cell motility shows promise as a method for in vitro assessment of cytotoxicity.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL SANDRA J. JOHNSON	22b. TELEPHONE (Include Area Code) (301) 671-2914 22c. OFFICE SYMBOL SMCCR-SPS-T

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

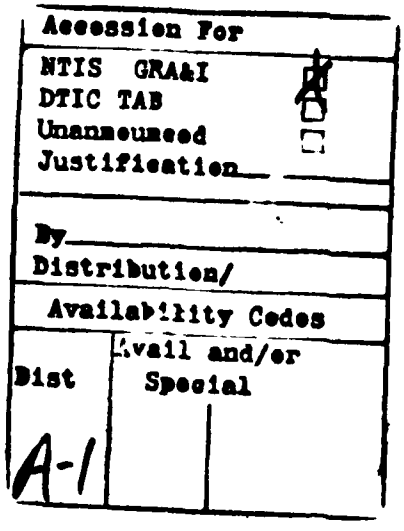
PREFACE

The work described in this report was authorized under Project No. 1L161101A91A, In-House Laboratory Independent Research. This report is a completed section of the project, and the experimental data are contained in laboratory notebook 87-0032. This work was started in April 1987 and completed in September 1987.

The use of trade names or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

In conducting the research described in this report, the investigators adhered to the 'Guide for the Care and Use of Laboratory Animals' as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities, and Care of the Institute of Laboratory Animal Resources, National Research Council.

This report has been approved for release to the public.



Blank

CONTENTS

Blank

CHEMICAL INHIBITION OF RABBIT SPERM CELL MOTILITY IN TOXICOLOGICAL TESTING

1. INTRODUCTION

Semen quality is commonly assessed by measuring sperm cell motility and velocity. Changes in motion characteristics occur during the structural and functional maturation of the spermatozoon. These changes appear to be concomitant with acquisition of fertilizing ability.[1-4] Many chemicals that induce changes in the swimming behavior of the sperm cell also inhibit fertilization. From a toxicological viewpoint, quantitation of changes in cell motion appears to be an attractive in vitro means of assessing the potential reproductive hazard of chemicals.[1] Because the absence of motion is usually equated with cell death, the motile sperm cell should also be suitable for assessing chemical cytotoxicity.[5] This report describes a study to determine the feasibility of using motile rabbit sperm cells as an in vitro screen for cell toxicity.

2. MATERIALS AND METHODS

2.1 Rabbit Housing.

New Zealand white rabbits were individually housed in standard rabbit cages in a room maintained at 25 + 2 °C. Humidity was maintained at 50 + 10%. Room lights, controlled by a timer, were maintained on a 12-hr light/12-hr dark cycle. Certified rabbit chow and water were available ad libitum.

2.2 Collection of Sperm.

Semen was collected with an artificial vagina.[6] The rest period between collection was at least 2 days. A log of semen volume and collection dates was maintained.

2.3 Sperm Cells Incubation.

Sperm cells were prepared for exposure to chemicals by washing the cells as described by Brackett, Brosquet, and Dressel[7] and resuspended in defined medium (DM).[8] The cells (1-3 x 10^7/mL) were suspended in DM; the test compound was added; and the suspension was incubated at 37 °C under an atmosphere that was 5% CO_2, 8% O_2, and 87% N_2. At timed intervals, a drop of the cell suspension was placed in a 10-μm deep Makler chamber. The chamber was set on the microscope stage in a stream of air heated to 37 °C. Sperm cells were observed using phase contrast microscopy (100x) to determine the cells' motility. Sperm cells were exposed to three concentrations of a test compound, usually 0.001M, 0.01M, and 0.025M. Because some of the compounds were not soluble in water, ethanol was selected as the solvent. When ethanol was

used, its concentration in the cell suspension did not exceed 0.25% (v/v), and an equal volume of ethanol was added to the control cells. Cells were considered nonmotile when no movement was detected in a minimum of five microscopic fields.

2.4 Test Compounds.

Methylphosphonofluoridic acid (MF), pinacolyl alcohol (PA), and methylphosphonic acid (MPA) were obtained from Mark Haley, Environmental Toxicology Branch, Research Directorate, U.S. Army Chemical Research, Development and Engineering Center (CRDEC). 2-Methylcyclohexanol, o-ethyl-o-(2-diisopropyl-aminoethyl)-methylphosphonite (QL), and dimethylmethylphosphonate (DMMP) were obtained from Dr. Eugene Olajos, Biosciences Branch, Research Directorate, CRDEC. Triethylphosphite (TEP) was obtained from Dr. H. D. Durst, Applied Chemistry Branch, Research Directorate, CRDEC. Ammonium oxalate, reagent grade, was a product of the Fisher Scientific Company (Silver Springs, MD). Oleic acid, reagent grade, was purchased from Matheson, Coleman, and Bell Chemical Company (Cincinatti, OH). Hydroquinnone was obtained from Chemical Service Company (Media, PA). Pyrogallol, reagent grade, was a product of J.T. Baker Chemical Company (Phillipsbury, NJ). Polyethylene glycol 200 (PEG 200) was obtained from Dr. J. James, Bioscience Branch, Research Directorate, CRDEC.

3. RESULTS

3.1 Sperm Cell Motion Characteristics.

Several types of time dependent motions were recognized. Immediately after washing and resuspension in DM, cells exhibited two types of motion characteristics: linear progression while rotating (A) and circular motion without rotation (B). Within 1 hr of incubation at 37 °C, in a 5% CO_2, 8% O_2, and 87% N_2 atmosphere, cells acquired a third type of motion. This motion (C) was characterized as a high velocity, rapidly rotating, linear progression. Types A and C usually predominated within the first hour. After 2-3 hr of incubation, three additional movement characteristics could be distinguished. These characteristics included: a rotational, linear progression associated with a side-to-side displacement of the head about the axis of progression, giving a jerky halting appearance to the cell's motion (D); rotational, linear progression with periodic changes in the directions of progression with or without a pause before the directional change (E); and a slow waveform of progression (F). Cells with types A, C, and D characteristics were most abundant after 4 hr of incubation. Between 4 and 6 hr of incubation, a small number of cells developed a highly active, wide displacement but nonprogressive type of motion, characterized by rapid side-to-side motion, bending of the neck piece, and wide curvature lashing movement of the tail. This particular combination of movements resulted

in the cell's head describing a stellar trajectory. This particular motion is referred to as hyperactivated motility in other species.[9] The percentage of sperm cells exhibiting hyperactivity, while low at 4-6 hr, was usually higher after overnight incubation. The percentage of sperm cells that developed hyperactivity varied between individual rabbits. Variation in percentage of hyperactivity among samples from the same rabbit was also evident. Some rabbit cells did not acquire hyperactivity.

3.2 Chemical Exposure of Sperm Cells.

All compounds studied induced changes in the character of cell motion and velocity of movement. Hydroquinone (HQ) and pyrogallol (PG) were the most toxic, inhibiting motility at a concentration of 0.001M less than 0.5 hr (Table 1). Polyethylene glycol had the least effect on sperm cells. They remained motile with reduced velocity after a 22-hr incubation. Pinacolyl alcohol appeared to be more toxic to sperm cells than PEG 200; fewer cells were motile after 22 hr of incubation at the same concentration (0.025M). Pinacolyl alcohol inhibited all movement within 1 hr at a concentration of 0.08M. Using inhibition of all motion as the end point, the compounds tested may be arranged in the following descending order of cytotoxicity: PG > HQ > oleic acid > ammonium oxalate > MF > methylcyclohexanol > methylphosphoric acid QL > DMMP > TEP > PA > PEG 200 (Table 1).

Table 1. Time (Hr) for Inhibition of Motility.

Compound	0.001M	0.01M	0.025M	0.0024M	0.024M	0.06M
PG	<0.5	<0.5	<0.5			
HQ	<0.5	<0.5	<0.5			
Oleic Acid	5.0-22.0	<0.5	<0.25			
Ammonium Oxalate	>22.0	>1.0	<0.5			
MPA		>4.0				
Methylcyclohexanol		>4.0*	<1.0**			
MF	>22.0	>22.0	>22.0			
QL	>22.0	>22.0	>22.0			
DMMP	>22.0	>22.0	5.0-22.0			
TEP	---	---	---	>22.0	<22.0	<1.0
PA	>22.0	>22.0	>22.0***			
PEG 200	>22.0	>22.0	>22.0			

*0.008M
**0.08M
***Nonmotile in 1 hr

4. DISCUSSION AND CONCLUSION

Cessation of cell motion is not a very sensitive end point in assessing the cytotoxicity of chemicals. Although sperm cells remained motile for several hours after exposure to the test compounds, there was a large variation between exposure groups in the number of motile cells present and their movement velocity. It is highly desirable to use an objective method when measuring percentage of cell motility, cell velocity, and cell motion characteristics. This would permit an objective, more accurate determination of a compound's cytotoxic potential. Table 2 lists the results of the Draize test conducted by J. Manthei and co-workers, Toxicology Division, Research Directorate, CRDEC, with several of the compounds. Agreement between the ranking of the Draize test and the order of inhibition of sperm cell motility was good, except for oleic acid. Oleic acid, while giving negative results in the Draize test, proved effective in inhibiting sperm cell motility. Table 3 lists the results of incorporating amino acid and uridine by mouse 3T3 cells in the presence of several compounds. This work was done by Dr. D. Starke, Rockefeller University (New York, NY). The agreement between the method of cytotoxicity assessment and sperm cell motility inhibition is also good. These preliminary results suggest that sperm cell motility inhibition may be useful as an in vitro procedure for the assessment of a chemical's cytotoxic effect. Measurements of changes in the percentage of motile cells, character of movement, and cell velocity would be more sensitive, increasing the procedure's use.

Table 2. Results of the Draize Test.

Compound	Effect
PG	Severe
Ammonium Oxalate	Severe
HQ	Moderate
PA	Moderate
DMMP	Mild
TEP	Mild
QL	----
Oleic Acid	----
PEG 200	----

Table 3. Concentrations of Chemicals for 50% Inhibition of Amino Acid and Uridine Uptake.

Compound	Amino Acid	Uridine
HQ	0.04	0.08
PG	0.07	0.52
TEP	0.40	0.50
Ammonium Oxalate	0.50	23.00
QL	6.10	41.00
PA	77.00	7.40
PEG 200	210.00	180.00

Blank

LITERATURE CITED

1. Yanagimachi, R., "The Movement of Golden Hamster Spermatozoa Before and After Capacitation," J. Reprod. Fertil. Vol. 23(1), pp 193-196 (1970).

2. Stein, D.M., and Schneider, H., "Effect of Antidepressant Drugs on the in vitro Egg Penetrating Ability of Golden Hamster Mesocricetus-Auratus Epididymal Spermatozoa," J. Reprod. Fertil. Vol. 68(1), pp 227-234 (1983).

3. Meizell, S., "Molecules that Initiate or Help Stimulate the Acrosome Reaction by Their Interaction with the Mammalian Sperm Surface," Amer. J. Anat. Vol. 174, p 285 (1985).

4. Bavister, B.D., Chen, N.F., and Fu, P.C., "Catecholamine Requirement for Hamster Sperm Motility in vitro," J. Reprod. Fertil. Vol. 56(2), pp 507-514 (1979).

5. Petzoldt, R., Wolf, H., Reefschlager, J., Karge, M., and Berger, G., "A Rapid Quantitative Method Based on Motility of Bull Sperm Cells for in vitro Toxicity Testing of Biomaterials," Biomaterials Vol. 6(2), pp 105-109 (1985).

6. First, N.L., "Collection and Preservation of Spermatozoa," In Methods in Mammalian Embryology, p 15, J.C. Daniels, Jr., Ed., Freeman, San Francisco, CA, 1971.

7. Brackett, B.G., Bousquet, D., and Dressel, M.A., "In vitro Sperm Capacitation and in vitro Fertilization with Normal Development in the Rabbit," J. Androl. Vol. 3(6), pp 402-411 (1982).

8. Brackett, B.G., and Oliphant, G., "Capacitation of Rabbit Spermatozoa in vitro," Biol. Reprod. Vol. 12(2), pp 260-274 (1975).

9. Yanagimachi, R., "Mechanisms of Fertilization in Mammals," In Fertilization and Embryo Development in vitro, L. Mastroianni and J.D. Biggers, Eds., Plenum Press, New York, NY, 1981.